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Viral and Non-viral Vectors in Gene Therapy: Technology Development and Clinical Trials

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Gene therapy as part of modern molecular medicine holds great promise for the treatment of both acute and chronic diseases and has the potential to bring a revolutionary era to cancer treatment. Gene therapy has been named the medicine of the future. For the past 10 years various viral and non-viral vectors have been engineered for improved gene and drug delivery. Although various diseases have been targeted, cancer therapy has been addressed to a large extent because of the straight forward approach. Delivery of toxic or immunostimulatory genes by viral and non-viral vectors has been investigated and encouraging results have been obtained in animal models. A large number of clinical trials have been conducted with some highly promising outcome. We propose that combinations of viruses with liposomes or polymers will solve the problem of systemic viral delivery and tumor targeting, bringing a revolution in molecular medicine and in applications of gene therapy in humans.

Introduction

Classic drug development has included a close interaction of various aspects such as biology, chemistry and pharmacology to engineer high efficacy medicines. The drawback with this approach is that the drugs are administered as such, as small molecule compounds or proteins at therapeutic concentrations, which will require readministration at defined intervals. In contrast, if the drug can be delivered by slow release in capsules or other mechanical devices or by gene delivery vehicles as nucleic acids a more sustained form of treatment is feasible. In the latter case, these vectors can be of either viral or non-viral origin and can provide, depending on which delivery system is applied, either short- or long-term heterologous gene expression.

Several key steps appear to be involved in effective gene transfer to somatic cells: (i) type of delivery vehicle that may be composed of cationic liposomes, other types of liposomes, polymers, and their combinations, various types of viral or hybrid vectors and combinations of viral vectors with polymers or lipids; (ii) interaction of the gene vehicle with serum components; (iii) its circulation time in body fluids and biodistribution; (iv) its escape from immune cells and macrophages; (v) its interaction with the surface of the cell; (vi) its triggering of apoptotic pathways by this interaction; (vii) its penetration through the cell membrane barrier; (viii) its release from endosomes or other subcellular compartments and its escape from degradation by intracellular nucleases; (ix) nuclear import; (x) ability of regulatory elements for driving the expression of the foreign gene in a particular cell type including DNA sequences that might determine integration versus episomal maintenance of a plasmid or viral vector; (xi) persistence of the plasmid in the nucleus (or of the virus) as an extrachromosomal element for many cell cycles or integration into active chromatin loci; (xii) main-

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tenance of expression for long periods; (xiii) passage to progeny cells, and (xiv) ability of the transcripts to be exported to the cytoplasm, translated, modified post-translationally and transported through the endoplasmatic reticulum and Golgi apparatus to the cell surface or extracellularly (1). Theoretically, a modest 2-fold enhancement in the efficiency of each one of the 14 steps described above would result in a 2^{14}-fold (about 16,000-fold) higher level of a therapeutic protein in the targeted cell.

It is therefore not surprising that a wide variety of delivery systems have been developed. Generally, these can be divided into two groups: viral and non-viral vectors. The viral vectors cover a wide range of viral species with different types of nucleic acid composition and characteristic features related to host cell specificity, expression pattern and duration as well as cytotoxicity (Table I). Generally speaking, viral vectors possess strong promoters, which are responsible for high-level heterologous gene expression. The nucleic acid content of each virus dictates to a large degree the features of gene expression. In this sense, viruses with single stranded RNA genomes, such as alphaviruses demonstrate a rapid onset of transgene expression, but also show their transient nature. DNA based vectors like adenovirus are also transient, whereas both adeno-associated viruses typically generate long-term expression due to their capability of integration into the host genome. Retroviruses, carrying a double-stranded RNA genome, can also establish long-term expression through chromosomal integration. Moreover, viral vectors based on herpes simplex virus, typically generate a latent infection, where the viral genome resides in host cells without causing any visible harm. Obviously, application of viral vectors for clinical trials in humans requires serious consideration of safety aspects related to their use.

In contrast to viral vectors, non-viral delivery systems possess a much reduced biosafety risk by nature. It is therefore not surprising that this area has been the target for intensive research and the development of a multitude of vehicles (Table II). Various liposome compositions have been attractive materials for non-viral vector development. Cationic liposomes as well as neutral/zwitterionic liposomes have been widely experimented. Additionally, polymer systems such as dendrimers, hyperbranched polymers and polymeric nanoparticles are under investigation. Coating of liposomes and polymers with polyethylene glycol (PEG) chains can significantly change the properties of liposomes, for instance protect the recognition by the host immune defense system. The two properties that severely has hampered the use of non-viral vectors have been the generally modest gene delivery efficiency and their short-term expression capacity. However, much research effort has been invested in the development of improved non-viral delivery methods.

In this review, both non-viral and viral vectors are described. Emphasis is put on various types of vectors developed and their possible applications. Additionally, alternative therapeutic genes are described. Also examples of the use of gene delivery vehicles in various clinical trials are presented.

Therapeutic Genes

The therapeutic efficacy is not only dependent on achieving excellent delivery of the therapeutic gene to the target tissue. It is also of great importance that the therapeutic gene has the wanted effect. For this reason, especially in cancer therapy most of the applied genes have been either of a cytotoxic nature, providing an efficient killing effect on the target cell or has an immunostimulatory effect, which

Table I
Viral Vectors

Vector	Genome	Packaging Capacity	Host Range	Features
AAV	dsDNA	low, < 4 kb	broad	slow expression onset genome integration long-term expression
Adenovirus	dsDNA	medium, < 7.5 kb	broad	transient expression strong immunogenicity
Alphavirus - SFV - Sindbis - VEE	ssRNA	medium, < 7.5 kb	broad	transient expression low immunogenicity
Herpes	dsDNA	high, > 30 kb	broad	latent infection long-term expression
Retrovirus	dsRNA	medium, 8 kb	restricted	long-term expression genome integration
Lentivirus	dsRNA	medium, 8 kb	broad	long-term expression genome integration
Poxvirus	dsRNA	high, > 30 kb	broad	transient expression
Baculovirus	dsDNA	medium	restricted	transient expression

Table II
Non-viral vectors

Vector	Composition	Features
Liposomes		
Cationic liposomes	DDAB	Toxicity, requires intraperitoneal transfer
	DODAP	
	DOGS	
	DOTAP	3-fold increase in human
	DOPE	colon cancer cells
Neutral / zwitterionic	Soy phosphatidyl choline	Extrusion through membranes
	Cholesterol	PEG coating
Polymers	Dendrimers	Form complexes with negatively charged
	Hyperbranched polymers	DNA
	Polymer nanoparticles	Targets ligands
Peptide-DNA Complexes	ppTG1-plasmid DNA	Destabilization of membrane
	PpTG20-plasmid DNA	

DDAB, dimethyldioctadecyl ammonium bromide; DODAP, 1,2-dioleyl-3-dimethyl-ammonium propane; DOGS, dioctadecylamidoglycylspermine; DOTAP (= DOTMA), N-(1-(2,3-dioleoyloxy) propyl)-N,N,N-trimethylammonium chloride; DOPE, 1,2-sn-dioleoylphosphatidylethanolamine

results in tumor regression. Below are summarized a few examples of commonly applied therapeutic genes.

Momentum of p53 Gene Therapy

A fascinating subject in cancer biology since its discovery has been p53 (2). Mutations in the p53 gene contribute to the emergence of the malignant phenotype. p53 monitors chromosome damage and either arrests cell-cycle progression or triggers apoptosis in cells with unrepaired lesions. The tumor suppressive activity of p53 seems to involve at least eight independent pathways: (i) Induction of the p21 gene which causes growth arrest both via inhibition of cyclin-dependent kinases and via inactivation of PCNA; PCNA is the accessory molecule to DNA polymerases α and δ and its absence causes arrest of DNA synthesis at the replication fork; (ii) Induction of the death-promoting *Bax* gene as a mechanism which eliminates oncogenic virus-infected and transformed cells; (iii) By a direct interaction of p53 with origins of replication preventing firing and initiation of DNA replication; (iv) Via binding of p53 to a number of important molecules involved in transcription (TATA box-binding protein or TBP, TFIIH); (v) p53 functions in DNA repair by patrolling the genome for small insertion deletion mismatches or free ends of DNA; (vi) p53 is able to attract RPA, an accessory to DNA polymerases α and δ as well as TFIIH and RAD51 at the damaged DNA sites; TFIIH, RAD51, and RPA have a demonstrated role in DNA repair; (vii) p53 induces Gadd45 involved in the arrest of the cell cycle and Mdm2, which after exceeding a threshold value in the cell associates with p53 to restrict its regulatory functions; thus, Mdm2 acts as a feedback loop for p53 to moderate its apoptotic and cell cycle restrictive functions; (viii) p53 specifically interacts with several viral proteins such as T antigen of SV40 and E6 of human papillomavirus (HPV). This interaction is thought to inhibit p53 functions during viral infection. A key event in cervical carcinogenesis is the disruption of p53 tumor suppressor pathway by HPV E6 oncogene. In over 50% of all human malignancies cells have lost this function of p53 because of mutations mainly in the DNA-binding region of the molecule. Transfer of the wild type (wt) p53 gene was able to suppress tumor cell proliferation. These studies initially in cell culture and in animal models were also applied to human clinical trials using adenoviral delivery locally to lung tumors.

E1A Gene of Adenovirus

The human adenovirus type 5 (Ad5) early region 1A (E1A) proteins have been shown to have potent antitumor effects, due to their ability to reprogram oncogenic signalling pathways in tumor cells. The E1A gene of adenovirus functions as a tumor inhibitor by repressing oncogene transcription; for example, E1A is a repressor of the Her2/neu tyrosine kinase gene at the level of transcription regulation and might thus have specific applications against breast and ovarian cancers overexpressing this oncogene (3). E1A also modulates gene expression resulting in cellular differentiation and induces apoptosis in cancer cells. Finally, E1A sensitizes cancer cells to chemotherapeutic drugs such as etoposide, cisplatin, and taxol. An adenovirus vector deleted of all viral protein coding sequences with the exception of E1A reduced the proliferative capacity of the human lung adenocarcinoma cell line A549, the ability of these cells to form colonies in soft agarose and gave a 10-fold greater sensitivity to the chemotherapeutic drug cisplatin (4).

Cancer Immunotherapy: Interleukin Genes and Immune Response Modulation

Several approaches are under development for cancer immunotherapy. Cytotoxic T-cells can recognize and kill tumor cells that present peptides derived from tumor-asso-

ciated antigens (TAA) on their surface when associated with major histocompatibility complex (MHC) class I molecules. However, immune responses to tumor-associated antigens are often suppressed by a tumor-induced state of immune anergy. Attempts to overcome tumor-induced T-cell anergy includes transfer of vectors carrying genes encoding one of a variety of cytokines (5). Monocyte-derived Dendritic Cells (DCs) from prostate cancer patients were transduced with recombinant adenoviral vectors and thus became more potent stimulators of allogeneic lymphocytes, produced increased amounts of the cytokines TNF-α and IL-12 p70, and exhibited increased expression of NF-κB and of the antiapoptotic molecules Bcl-X_L and Bcl-2 (6). T-cell-based immunotherapies provide a promising means of cancer treatment. However, the shortcomings of a durable antitumor response arise from a lack of trafficking of specific T-cells to tumors. Chemokines such as Gro-α (Growth-Regulated Oncogene-α; CXCL1) and RANTES (Regulated on Activation Normal T-Cell-Expressed and Secreted; CCL5) are expressed and secreted by a range of tumors including fine-needle aspirates of melanoma from patients and may serve as suitable targets for redirecting the migration properties of specific T-cells toward tumor. Transduction of T-cells with a retroviral vector carrying the CXCR2 receptor for Gro-α made them responsive to Gro-α and induced interferon-γ (IFN-γ) secretion (7).

B lymphocytes are also attractive targets for gene therapy of genetic diseases associated with B-cell dysfunction and for immunotherapy. Onco-retroviral and HIV-derived lentiviral vectors, pseudotyped with ecotropic, amphotropic or vesicular stomatitis virus (VSV-G) envelopes, have been used for transduction of human and mouse B lymphocytes; VSV-G pseudotyping facilitated gene transfer into all cell types (8). A single intratumoral administration of an adenoviral vector carrying the mouse RTVP-1 gene (Related to Testes-specific, Vespid, and Pathogenesis proteins) significantly reduced primary tumor growth as well as spontaneous metastasis to lung in an aggressive model of prostate cancer. The mechanism included increased apoptosis, lower microvessel density counts by inhibition of endothelial cell sprouting as well as an increase in the infiltration of tumor-associated macrophages, dendritic cells, and $CD8^+$ T-cells in treated tumors. This gene seems to have a potential in immunotherapy of cancer (9).

Polyclonal stimulation of T-cells, preferably via the TCR complex, results in a cascade of cytokines associated with T-cell activation to overcome T-cell anergy. Intratumoral injection of a highly attenuated MVA poxvirus expressing antibodies specific for the CD3ε chain (KT3) in animal models induced activation of immune effector cells and resulted in rejection of the tumor. Furthermore, the combination of MVA-KT3 with Ad expressing the IL-12 or IL-18 cytokines as well as the RANTES, MIP1β chemokines caused tumor rejection (5). The muscle as a target tissue for somatic gene therapy by particle-mediated intramuscular gene-gun transfection has been explored in a rat sarcoma model; using a combination of IL-12 and IL-18 cDNA significant levels of IFN-γ, a high activity of tumor-specific cytotoxic T lymphocytes and long-term antitumor immunity were induced by this approach (10).

Inhibition of Cancer Cell Proliferation and Invasion with Genes

Uteroglobin is a secretory protein with anti-inflammatory properties synthesized by most epithelia, including the respiratory tract and is related to inhibition of phospholipase A2. Adenovirus-mediated uteroglobin gene transfer has been exploited and was effective in arresting growth of lung cancer cell lines that did not express the uteroglobin gene (11).

Antisense Oligodeoxynucleotides (ODNs) and Ribozymes

The field of ODNs has been developed in a sophisticated manner and novel pharmaceutics appear to emerge based on ODNs. Evidently, several approaches using ODNs could be done at the gene therapy level using the ODN genes. This will reduce cost, toxicity and ensure the presence of steady state levels of a therapeutic ODN in the cytoplasm or nucleus. Combinations of ODNs that targeted different regions of the thymidylate synthase mRNA were able to inhibit cell proliferation. This strategy can also be used in combination with the thymidylate synthase-directed chemotherapeutic drugs raltitrexed and 5-fluorodeoxyuridine (12).

Novel avenues for anticancer therapeutic intervention include the development of hammerhead ribozymes or employment of oligonucleotides designed to cleave specific mRNAs and thus diminish the levels of a protein in a tumor cell. For example, suppression of the gene expression multidrug resistance 1 (MDR1) can reverse multidrug resistance to doxorubicin and etoposide (although not to cisplatin) in cell cultures (13). Suppression of the multidrug resistance-associated protein (MRP) and multidrug resistance 1 (MDR1) gene expression in HCT-8DDP human colon cancer cell lines using hammerhead ribozymes, designed to cleave the MRP and MDR1 mRNAs, was sufficient to reverse multidrug resistance to doxorubicin and etoposide (VP-16) but did not affect resistance to cisplatin, methotrexate and 5-fluorouracil (14).

Survivin, a novel apoptosis protein expressed in most human carcinoma cells but not in normal tissue could be downregulated in MCF-7 breast cancer cells by two hammerhead ribozymes (RZ-1 and RZ-2) (15). Applying adenovirus-based delivery the survivin mRNA reduction was 74%, which resulted in increased apoptosis and suggested that the ribozyme approach was feasible for cancer therapy.

Immunization with Tumor Antigens

Another therapeutic and also prophylactic approach has been to apply either nucleic acids or viral vectors for vaccination against tumor antigens. Basically, the gene coding for the tumor antigen of interest is inserted in a mammalian expression plasmid, which is then systemically (intravenously or intraperitoneally) administered into various animal models (16). Plasmids carrying the alphavirus replicon has proven to be highly efficient resulting in improved immune responses with lower DNA concentrations compared to conventional plasmid vectors (17). Additionally, injection of naked RNA has proven efficient for the generation of strong immune responses (18). In this context, a single intramuscular injection of an alphavirus RNA replicon carrying the bacterial β-galactosidase gene resulted in protection against tumor challenge of mice and prolonged the survival time of animals with established tumors (19). The administration of recombinant viral particles expressing various viral and tumor antigens has also protected against lethal challenges with viruses or tumors (20). Studies have been conducted in various animal models including mice, guinea pigs and primates.

Viral Vectors

Typically, viral vectors consist of viral particles with nucleic acid covered by at least a capsid protein and in many cases further by an envelope structure. Generally, one or several viral structural genes are deleted to disable infectious particles from spread in the host organism. Viral vectors typically contain strong promoters to support high level of transgene expression. Viral DNA vectors can also include tissue-specific promoters to exclude expression in another places than the target tissue. Other means of targeting include the possibility to introduce specific recognition sequences in the surface structures of the virus coat to enable infection of specific cells/tissue.

In addition to disable viruses from replicating in host cells and producing progeny, it has turned out that deletion of viral genes has significantly reduced the cytotoxicity on host cells and therefore improved the host cell survival. Even in the case of cancer therapy, this might be advantageous, since a prolonged host cell survival will also allow improved transgene expression and therefore a better therapeutic efficacy.

Several viral vectors, such as adeno-associated viruses (AAV) and retroviruses, can integrate into the host chromosomes and thereby generate a permanent genome presence in the host cells resulting in long-lasting expression of the gene of interest. Recent discoveries have, however, revealed that the integration site is crucial and especially integration into regions of active genes and particularly oncogenes, can result in devastating effects (21). Below are described recent progress for various viral vectors and some insight into clinical applications.

Adeno-associated Viruses

Today, AAV vectors have become increasingly popular mainly because of their broad transduction range in tissues such as liver, muscle, retina and the central nervous system and for their long-term expression mode (22). The limited packaging capacity and the relatively inefficient large-scale production of AAV has restricted the use of these vectors to some extent. Other issues of concern for application of AAV are the preexisting immunity in humans to AAV and the random integration into the host genome. Various AAV serotypes have shown significant differences related to cellular entry and expression patterns and for instance AAV1 has demonstrated strong expression in skeletal muscle and retina, whereas AAV5-based expression is more neuronal and lung specific (23). As AAV2 shows long-term albeit poor expression, a recently discovered novel AAV serotype, AAV8, isolated from rhesus monkeys generated a 100-fold higher factor IX expression in liver cells, which was not compromised by preimmunization with other AAV serotypes (24).

Moreover, AAV vectors have been used for *ex vivo* transduction of B cells from chronic lymphocytic leukemia (CLL) patients (25). Infection with AAV-CD40L virus at a multiplicity of infection (MOI) of 100 resulted in transduction rates of 97% and up-regulation of CD80 not only in infected cells but also in non-infected bystander leukemia B cells and could provide a promising vaccination strategy for patients with B-CLL. In another application a mutant interleukin-4 receptor antagonist (IL-4RA), which can inhibit IL-4 an IL-13, crucial components in asthma pathogenesis, was delivered to the airways of mice by AAV vectors (26). This treatment reduced airway hyperresponsiveness and IL-4 and IL-13 triggered airway eosinophilia and therefore presents a potential option for control of chronic airway inflammation and asthma. Muscle injection of AAV expressing the α-galactosidase A, a lysosomal enzyme responsible for Fabry disease, in Fabry knock-out mice was examined as a possible replacement therapy (27). The α-gal A activity in plasma increased to 25% of normal mice and remained at elevated levels for at least 30 weeks. AAV vectors have also been used for gene delivery to the eye (28). Efficient gene delivery to photoreceptors and pigment epithelial cells was observed. However, targeting retinal ganglion cells (RGCs) has been more problematic. Using a modified AAV vector with a chicken β-actin promoter and a post-transcriptional regulatory element from woodchuck hepatitis virus 85% transduction rates of RGCs was observed within 2 weeks after a single intravitreal injection.

Adenoviruses

Adenovirus vectors are probably the most frequently used viral vectors, mainly because of the engineering of packaging cell lines for the generation of high-titer virus stocks and the high-

level gene expression obtained in a broad range of host cells. The early vesions of adenovirus vectors showed toxic effects on host cells and typically induced strong immune responses. However, second- and third-generation vectors in which several non-essential viral genes have been deleted demonstrated reduced toxicity and improved gene expression (29).

A heat-directed suicide gene therapy strategy based on an adenoviral vector (Ad.70b.CDTK) has been described. The prodrug-activating *E. coli* cytosine deaminase/herpes simplex virus thymidine kinase (CDTK) fusion protein was expressed under the control of the hsp70b promoter. As a result, treatment of breast cancer cells at 43° C in the presence of the prodrugs 5-fluorocytosine and ganciclovir resulted in 30- to 60-fold decreases in clonogenic survival (30). In another study, two separate adenovirus vectors expressing the *E. coli* CD and uracil phospohoribosyltrasnferase (UPRT) genes were used in combination with 5-FC administration (31). Intratumoral injections of nude mice with both adenovirus vectors significantly suppressed the tumor growth. Adenovirus vectors have also been applied for bladder cancer therapy (32). It was demonstrated for the first time that human transitional cell carcinoma (TCC) biopsies were transfected as efficiently as intact normal urothelium.

Application of replication-deficient adenovirus vectors for brain tumors has been disappointing, mainly due to the inefficiency of transducing glioma cells and the limited spread of the vector in the tumor. The use of replicating adenovirus vectors has the potential of overcoming these limitations (33). A number of replication-selective oncolytic adenoviruses designed to replicate selectively in tumor cells by targeting molecular lesions inherent in cancer, or by incorporation of tissue-specific promoters driving the early genes that initiate viral replication, are currently under clinical evaluation, also referred to as virotherapy (34). Oncolytic adenovirus therapy shows the best results and achieves an enhanced tumoricidal effect when used in combination with chemotherapeutic agents such as cisplatin, leucovorin and 5-fluorouracil. Improvement of oncolytic adenoviruses is directed at molecular engineering tumor cell-specific binding tropism, selective modifications of viral early genes and incorporation of cellular promoters to achieve tumor-specific replication, augmentation of anti-tumor activity by incorporation of suicide genes, and manipulation of the immune response (35). Replication-activated adenoviral vectors have been developed to express a secreted form of β-glucuronidase and a cytosine deaminase/uracil phosphoribosyltransferase. β-glucuronidase activates the prodrug 9-aminocamptothecin glucuronide to 9-aminocamptothecin. The cytosine deaminase/uracil phosphoribosyltransferase activates the prodrug 5-fluorocytosine to 5-fluorouracil (5-FU) and further to 5-fluoro-UMP. The combination of this adenoviral vector with prodrug therapy enhanced viral replication and its spread in liver metastases derived from human colon carcinoma or cervical carcinoma in a mouse model (36).

Alphaviruses

Alphavirus vectors have been efficiently used for *in vitro* and *in vivo* transgene expression and vaccine production (37). The most commonly used expression vectors are based on Semliki Forest virus (SFV) (38), Sindbis virus (SIN) (39) and Venezuelan equine encephalitis virus (VEE) (40). Generally, expression systems based on alphavirus vectors are engineered to contain an expression vector carrying the viral non-structural genes and a helper vector accommodating the structural (capsid and envelope) genes (41). The presence of the nonstructural genes is essential as their gene products form the replicase complex responsible for highly efficient RNA replication (200,000 fold) in the cytoplasm of the infected cells. This will result in high-level transient expression and induction of apoptosis. The transient nature of expression and limited spread of replication-defective SFV particles has been demonstrated in rat brain (42).

Alphavirus vectors have been frequently used for vaccine production in animal models applying vectors as nucleic acids (naked RNA, DNA plasmids) and recombinant particles (37). By this procedure, immunization resulted in protection against challenges with lethal viruses and tumors. Typically, tumor protection was obtained in mice after interleukin-12 (43) and B16 and 206 glioma overexpression (44) from SFV particles. Moreover, immunization of mice with SFV particles carrying the P1A gene led to protectction against challenges with P185 antigen (45). Additionally, mice vaccinated with VEE containing the E7 gene from human papilloma virus Type 16 prevented new tumor formation (46).

SFV vectors have also been used for studies in animal models for intratumoral injections. It was demonstrated that injection of SFV particles expressing the p40 and p35 subunits of interleukin-12 (IL-12) generated tumor regression and inhibition of tumor blood vessel formation in a B16 mouse melanoma model and repeated injections enhanced the anti-tumor response (47). In another study, intratumoral injection of SFV particles expressing IL-12 resulted in significant tumor regression (43). The size of the treated tumor affected the therapeutic outcome as lower efficacy was observed for large tumors. The IL-12 expression also generated long-term immunity as tumors did not reoccur in treated animals even after rechallenge with the tumor. Furthermore, intratumoral injection of SFV-GFP particles into nude mice implanted with human lung carcinoma resulted in rapid decrease in tumor volume compared to control animals (48). The best results were obtained after 3 injections on consecutive days followed by another 3 injections one week later. Another study in nude mice demonstrated

that SFV-based expression of the pro-apoptotic gene Bax reduced the growth of AT3-Neo and AT3-Bcl2 tumors (49).

Systemic delivery of alphavirus particles has been of major concern because of the broad host range and especially the strong preference of neuronal expression (37). To overcome this problem, preliminary targeting of Sindbis virus has been obtained by introducing IgG binding domains of protein A in the E2 envelope protein (50). The chimeric Sindbis virus showed a substantial reduction, 10^5-fold, in infection rates of BHK while cells treated with a monoclonal antibody against a surface marker protein became susciptible to Sindbis virus through the protein A domains. Another approach to achieve targeting was investigated by encapsulation of SFV particles in liposome structures, described in more detail below (51).

Herpes Simplex Viruses

Typically, Herpes simplex virus (HSV) vectors have been attractive for their large capacity to accommodate foreign DNA and their lifelong episomal presence in the latent phase in the host organism. The use of HSV vectors for gene therapy applications has, however, been hampered by the cytotoxicity especially observed in neurons. This obstacle has been resolved by deletion of non-essential HSV genes, such as several immediate early (IE) genes. For instance, removal of the ICP0, ICP4, ICP22 and ICP47 generated a completely non-cytotoxic HSV vector, which persisted for extended time in the nervous system of the host (52).

HSV vectors are particularly attractive for treatment of neurological disorders where long-term expression of therapeutic genes are required. Expression of nerve growth factor (NGF) from HSV vectors with latency active promoter (LAP) led to protection of dorsal root ganglion neurons from oxidative insult by H_2O_2 in the peripheral nervous system (PNS) (53). A prophylactic effect was also observed as sensory neurons were protected from toxic insult. In another application a conditionally replicating HSV vector was used for prepro-enkephalin expression to target chronic pain, which resulted in processing and release of native prepro-enkephalin (54).

Replication-defective HSV vectors have also been used for targeting muscular delivery. Because HSV has the capacity to accommodate large inserts, they are suitable for treatment of hereditary muscular diseases such as various forms of muscular dystrophy. A triple IE (ICP4, ICP22, ICP27) mutant HSV vector with substantially reduced cytotoxicity was employed to express dystrophin in dystrophin-null myotubes, which resulted in dystrophin-null muscle although mainly close to the injection site (55). Mutant IE HSV vectors were also applied on monkey $CD34^+$ stem cells, which after transplantation into monkeys with skin autographs generated reporter gene expression for more than 3 months (56).

Retroviruses

The prototype retroviruses are murine leukemia viruses (MLV), which has the capacity of integrating into the host cell genome and therefore provide presence in progeny cells and long-term expression. Although retroviruses present a rather broad host range their failure to infect non-dividing cells such as neurons, has to some extent restricted their applicability. One approach to overcome this limitation has been to pseudotype retroviruses with envelopes from other viruses such as the G glycoprotein from Vesicular Stomatitis virus (VSV) (57). In addition to the broader host range VSV-G pseudotyped retroviruses are less labile and may be concentrated to high titers. Much attention has been paid to the development of self-inactivating and self-activating vectors, which has been achieved by introduction of directly repeated sequences leading to high-frequency deletions during reverse transcription (58). Retrovirus vectors have been applied for cancer therapy and bone marrow transplantation. Typically introduction of retrovirus particles expressing the HSV-tk gene and administration of ganciclovir showed high efficiency in treatment of graft-versus-host disease (59). Newly generated replicative retrovirus vectors have been compared to defective counterparts for transduction efficiency in immunocompetent mice and were shown to transduce more than 85% of tumor, whereas the defective vectors transduced less than 1% (60). Moreover, the replicative vectors were 1000 times more efficient than adenovirus vectors.

The most exciting application of retroviruses has so far been the attempts to correct the SCID-XI phenotype in infants (61). Although full recovery was obtained for several patients recent observations that a leukemia-like state was developed in two infants has brought forward the potential risks of gene therapy (1). Linear amplification-mediated PCR analysis indicated that the retrovirus had integrated into the LMO2 gene, associated with childhood cancer.

Lentiviruses

Lentiviruses belong to the retrovirus family, but have some special features such as the capacity to transduce non-dividing cells. Many of the lentivirus vectors used in gene therapy applications are based on human immunedeficience viruses (HIV) or related viruses such as simian immunedeficiency viruses (SIV) or feline immunedeficiency viruses (FIV) (62, 63). Additionally, equine infectious anemia viruses (EIAV) have been applied because of their high capacity to transduce human cells without the possibility to replicate in cells of human origin, which obviously sets enhanced safety standards (64). Recently developed lentivirus vectors have resulted in efficient transduction of hepatocytes (65). High transduction delivery rates of a reporter gene was demonstrated in rat hepatocytes *ex vivo* and intravenous

administration in SCID mice led to 30% transduction of parenchymal and non-parenchymal liver cells. Targeted expression in liver cells could be achieved by using an albumin promoter, which resulted in therapeutic levels of factor IX for over one year after a single injection.

The transduction rates on ovarian cancer cells of a reporter gene were evaluated for lentivirus and retrovirus vectors (66). The gene transfer was similar for the two types of viruses in ovarian cell cultures. However, the transduction rates were much higher in growth-arrested cells for lentiviruses. Moreover, the lentiviral vector delivered the GFP reporter gene 10-fold more efficiently in SCID mice. Lentiviral vectors have been highly efficient for long-term expression in the brain *in vivo* (67). The expression of the glial cell line-derived neurotrophic factor (GDNF) in a nonhuman primate model for Parkinson's disease after injection into striatum and substantia nigra showed encouraging results. The long-term expression led to recovery of functional deficits and completely prevented the striatal degeneration.

Poxviruses

Poxviruses are a very large family of dsDNA viruses that can infect a broad range of mammals, birds and insects. However, only two human poxviruses have been identified and in principle they only infect human cells, although limited infections can occur by for instance avian poxviruses. The poxviruses are large and their replication is exceptional for DNA viruses as it occurs in the cytoplasm. Especially Vaccinia virus has been subjected to the engineering of non-replicating expression vectors (68).

Recently, tumor-selective replicating Poxvirus vectors have been developed for a mutated WR strain of vaccinia virus, which showed a high efficiency of *in vivo* replication. Intradermal injection of 10^6 pfu of the wild type (non-mutated) vaccinia in non-human primates caused a dermal zone of necrosis directly related to cellular destruction from viral replication. A mutant virus containing insertional deletions of both the thymidine kinase (TK) and vaccinia growth factor (VGF) genes no longer caused destruction of normal tissue, but had completely preserved replication efficiency in tumor tissue and could be safely delivered systematically to successfully treat subcutaneous tumors in mice (69). Recombinant poxviruses as vectors for in situ tumor transfection with immune-enhancing cytokines and immune co-stimulatory antigens might enhance immune recognition of tumors inducing an effective systemic antitumor immune response (70).

Cancer vaccinations based on the modified Vaccinia virus Ankara (MVA) were conducted for the expression of the human tyrosinase, which is a melanoma-specific differentiation antigen (71). This approach resulted in an efficient tyrosinase- and melanoma-specific Cytotoxic T-Cell (CTL) response using MVA-hTyr-infected dendritic cells. Immunization of transgenic mice induced CTLs specific for the hTyr-derived peptide (epitope 369-377) and the *in vivo* primed CTLs recognized and lysed human melanoma cells. This study is a good basis for conducting MVA-based vaccination of cancer patients.

Baculoviruses

Baculovirus vectors have been commonly used for recombinant protein expression in insect cells (72). Recently, modified Baculovirus vectors were demonstrated to also infect mammalian cells when applied at high virus concentrations. Due to their incapacity of replicating in human cells, Baculovirus vectors were therefore considered as safe for gene therapy applications. The transduction efficiency and biodistribution of Baculovirus vectors in rat brain was investigated (73). Baculovirus specifically transduced cuboid epithelium of the choriod plexus in ventricles at a high freqency (76%). The microglia response was only modest and nested RT-PCR analysis indicated that some expression was observed in the hindbrain and in other organs such as spleen, heart and lung.

Non Viral Vectors in Gene Therapy

The clinical utility of gene therapy systems has been impeded from inefficient transduction of current gene transfer vectors, limited output from expression cassettes, and loss of expression. Novel gene therapy systems based on liposomes are emerging as powerful gene delivery vehicles promising to overcome hurdles of gene therapy applications in a clinical setting.

Liposomes in Gene Therapy

In developing non-viral vectors, the goal is to design a system that simultaneously achieves high efficiency, prolonged gene expression and low toxicity. Synthetic methods use cationic lipids, polymers or proteins that complex with DNA, thus condensing it into particles of size 100 to 300 nm. One of the main goals is to identify the important chemical and physical parameters (i.e., the lipid structure, its charge distribution, or/and the mechanical properties of the cell membrane) and determine how they correlate with transfection. Among the obvious parameters needed for achieving efficient transfection is the requirement that the interaction of DNA with the vectors should yield complexes of small size, close to that of viruses. Another goal is to design particles that can target the DNA to particular cells. This may be achieved by adding ligands on the particle surface. There have also been attempts to combine the benefits of viral and non-viral systems into one delivery vehicle. It is highly likely that the vectors of the future will be hybrids of viral and non-viral formulations.

Cationic Liposomes

Cationic lipids are usually dissolved in $CHCl_3$, made into a dry thin film in a round glass flask by rotary solvent evaporation, and hydrated with water. Cationic liposomes can then be prepared by sonication using a water bath sonicator or probe sonicator. Cationic lipids may include DDAB, DODAP, DOGS, DOTAP, DC-Chol in 1:1 molar ratios with the zwitterionic and fusogenic DOPE. [Abbreviations: DDAB: dimethyldioctadecyl ammonium bromide (same as N,N-distearyl-N,N-dimethylammonium bromide); DODAP: 1,2-dioleoyl-3-dimethylammonium propane; DOGS: Dioctadecylamidoglycylspermine; DOTAP (same as DOTMA): N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride; DC-Chol, 3β-(N-(N´,N´-dimethylaminoethane) carbamoyl)cholesterol. DOPE, 1,2-sn-dioleoylphoshatidylethanolamine.] Cationic lipids can be used in clinical trials but because of their toxicity for intravenous infusions they have been proposed for intraperitoneal transfer of genes such as in intraperitoneally metastatic ovarian cancer (74). Using a lipoplex consisting of tetradecylphosphocholine/ dimethyldioctadecylamine/cholesterol/dioleylphosphoethanolamine liposomes a 3-fold increase in transfection efficiency was obtained in human colon carcinoma cells (75).

One of the most promising approaches has been the development of Allovectin-7, which consists of a DNA plasmid harboring the genes for the allogenic MHC class I protein, HLA-B7 and β2-microglobulin and complexed with a cationic lipid mixture (76). This DMRIE/DOPE lipid mixture facilitates the DNA uptake to tumor cells. As the HLA-B7 is infrequently expressed especially in the US population it permits an allogeneic immune response in most patients (77). The β2-microglobulin gene was introduced into Allovectin-7 to compensate for potential deficiency in MHC class I expression in tumor cells (78). Allovectin-7, therefore, provides several potential immuno-stimulating functions and has been subjected to Phase I clinical trials as described below.

Neutral/zwitterionic Liposomes

In addition to cationic liposomes the use of neutral/zwitterionic liposomes for delivery of DNA to cells has been investigated (79). The neutral/zwitterionic liposomes composed for example of soy phosphatidyl choline and cholesterol may be prepared by a variety of methods such as extrusion through membranes of pore sizes of 0.2 μm, to 0.05 μm under pressure in ultrapure nitrogen. About 15 passages need to be used to break liposomes down to an average particle diameter of 80 nm in order to achieve long circulation properties. The size distribution of a liposome preparation can be measured at a 90° angle with dynamic light scattering using for example the N4+ nanoparticle analyzer of Beckman-Coulter. A PEG coating can be provided to the liposomes by including the methoxy-polyethylene glycol–distearoyl phosphatidyl-ethanolamine lipid conjugate (mMPEG2000-DSPE) in the lipid mixture.

Polymers

A variety of polymers systems including dendrimers, hyperbranched polymers, polymeric nanoparticles are under development. Cationic functionalization (for example, introduction of quaternary ammonium groups) endows polymers with the property to form complexes with negatively charged DNA. Targeting ligands may also be introduced on the surface of the dendrimers and hyperbranched polymers (e.g., guanidinium groups, folate, galactose, manose, etc.).

Dendrimers are nanometer-sized, highly branched and monodisperse macromolecules with symmetrical architecture, while hyperbranched polymers are of similar structure but less symmetric and polydisperse. They both consist of three distinct segments, i.e., the central core, the branching units and the terminal functional groups. The core together with the internal units determine the microenvironment of the nanocavities and, in turn, their solubilization properties, whereas the nature and the number of the external groups characterize their solubility, chemical and biological behavior.

Nanoparticles have been used in diversified ways to deliver drugs and genes into cells. When suitably encapsulated, a pharmaceutical can be delivered to the appropriate site, its concentration can be maintained at proper levels for long periods of time, and it can be prevented from undergoing premature degradation. By reproducibly attaching targeting ligands to the nanoparticles, the drugs or other molecules can primarily be directed to their desired sites of action. Chitosan, a natural cationic polysaccharide, biodegradable poly (D,L-lactic acid) and poly(L-lysine)-graft-polysaccharide and thermosensitive copolymers based on poly(N-isopropylacrylamide) are under development (80).

Peptide-DNA Complexes

Moreover, basic amphiphilic peptides have been designed as components to bind to nucleic acids as means of destabilizing liposomes and therefore improving plasmid delivery to cells. Two 20 amino acid peptides, ppTG1 and ppTG20, complexed with plasmid DNA led to improved transfection of various murine and human cell lines (81). Additionally, the first successful gene transfer in live animals was obtained for a single-component peptide vector, which was demonstrated by luciferase expression in the mouse lung after intravenous injection. The efficient gene transfer by the ppTG1 and ppTG20 peptides was based on structure-function studies most likely due to their ability to exist in an α-helical confirmation.

PEGylation of Liposomes

"PEGylation", i.e., coating the surface of liposomes or polymer nanoparticles with polyethyleglycol chains (PEG) can give to a drug or gene delivery vehicle unique characteristics. Coating the surface of liposomes with inert materials designed to camouflage the liposome from the body's host defense systems was shown to increase remarkably the plasma longevity of liposomes, in a way similar to the erythrocyte coated with a dense layer of carbohydrate groups to evade the immune system (82). The brain tissue-derived ganglioside GM_1 (83) or phosphatidylinositol (84) enhanced circulation time of liposomes in the blood stream. PEG modification had been used for many years to prolong the half-lives of biological proteins (such as enzymes and growth factors) and to reduce their immunogenicity (85). PEG-coated liposomes circulated for remarkably long times after intravenous administration (86-88). Whereas the half-life of antimyosin immunoliposomes was 40 min, their coating with PEG increased their half-life to 1000 min after intravenous injection to rabbits (89). Both Lipoplatin and Lipogenes with a PEG coating on their surfaces are endowed with long circulation properties. Lipoplatin (cisplatin encapsulated into liposomes) has a half-life of 36 h in patient's sera (90).

Combinations of Viruses with Liposomes

Several limitations and drawbacks have been linked to adenoviral gene delivery such as a pre-existing immunity in patients and the generation of strong immune responses after re-administration of viral vector (although it has greatly been improved with the advent of gutless adenoviral vectors). Another setback arises from the inability of systemic delivery and most applications have used local (intratumoral) injection of the gene vector. Indeed, the lack of a targettable viral construct, encoding for example genes for toxins and/or prodrugs, able to localize to all tumors following systemic administration has proven to be a major limitation in their use for metastatic disease. This is not the final solution to cancer; indeed, over 90% of cancer patients succumb because of complications of metastases and not from complications from the primary tumor. Treating all micrometastases in an advanced stage patient by local delivery is practically impossible; therefore, systemic delivery is a key solution against cancer. Adenoviruses and most other viruses cannot be delivered systemically because they elicit an immune response.

Future developments might be directed in combinations of viruses with nonviral systems. Liposomal encapsulation of viruses allows systemic delivery. Liposome nanoparticle entrapment of a virus hides it from the immune system and makes its systemic delivery feasible without eliciting an immune response that would lead to the destruction of the viral particles-carriers of the therapeutic gene. Furthermore, using tumor-targeted liposomes the entire virus-liposome complex will be able to passively concentrate into solid tumors and metastases after intravenous injection into cancer patients. This system promises to solve major hurdles in gene therapy. The feasibility of the systems in SCID mice with human cancers as animal models has been tested using transfer of the β-galactosidase gene (91).

In another study, adenovirus particles were encapsulated using bilamellar DOTAP: chol liposomes (92). These procedures should eliminate the problem of generating humoral immune responses against adenovirus particles when readministered and also limit the requirement of appropriate receptors on the target cells. Efficient encapsulation of adenovirus was demonstrated by electron microscopy and transduction of Ad-LacZ of otherwise adenovirus resistant cells was achieved. The encapsulated particles were also resistant to the neutralizing effect of human anti-adenovirus antibodies *ex vivo* and *in vivo*.

LipoVIL12

LipoVIL12 is an encapsulated SFV carrying both p35 and p40 subunits of the human gene and expressing a functional and secreted IL-12 with a potential in immunotherapy of cancer, in restricting growth in tumor vasculature but also against viral and other infections. The phase I clinical trials with LipoVIL12 were performed as a second or third line treatment against advanced melanoma and kidney carcinoma refractory to other treatments using escalating doses of 10^8 to 5×10^9 encapsulated virus particles/m^2 (Boulikas *et al*., in preparation). These studies have established the MTD (maximum tolerated dose) as 3 billion encapsulated virus particles/m^2. There was no kidney, liver, bone marrow, neuro-, or cardio-toxicity or any other toxicity observed, except allergies. There were no side effects arising either from the virus or the liposome capsule at any dose and the observed allergies were arising from the overproduction of the therapeutic protein IL-12 concomitant with an increase in tumor necrosis factor-α and IFN-γ levels as shown by ELIZA tests on patient's sera.

Other Systems

Endovascular microcoils used in interventional procedures to treat cerebral aneurysms have been successfully used as a gene delivery system. Anti-adenoviral monoclonal antibodies were covalently attached to the collagen-coated surface of either platinum or polyglycolic acid microcoils and used to tether replication-deficient adenovirus encoding green fluorescent protein or β-galactosidase on animal models (93).

Combination of Gene Therapy with Chemotherapy and Radiotherapy

An emerging concept is that combinations of gene therapy regimens with chemotherapy has synergistic antitumor effects. IFN-β inhibits cell cycle progression, which mainly occurs as S phase accumulation. Pretreatment of tumor cells with IFN-β could significantly potentiate the cytotoxicity of cisplatin, 5-FU, paclitaxel and gemcitabine in cell cultures (94). Evidently, combination of IFN-β gene transfer with certain antineoplastic drugs is expected to have a synergistic effect in cancer treatment. Platinum-based chemotherapy enhances mutations in the p53 in the heterogenous tumor cell population; transfer of the wild type p53 gene enhances the sensitivity of chemoresistant cells to cisplatin and cisplatin-induced apoptosis (95).

Interleukins potentiate platinum drug cytotoxicity and can also be used to improve bone marrow function. Cisplatin and IL-1 treatment induced a blockade at G1/S of the cell cycle, down-regulating c-myc gene and inducing p53-dependent apoptosis in ovarian carcinoma cells (96). Therefore, transfer of the IL-1 gene would potentiate platinum drug cytotoxicity and is anticipated to have a significant potential in cancer treatment. Recombinant human interleukin-3 (rhIL-3) shortened the duration of chemotherapy-induced neutropenia and thrombocytopenia; concurrent administration of rhIL-3 and of a chemotherapy regimen for relapsed small cell lung cancer (vincristine, ifosfamide, mesna, and carboplatin on day 1 every four weeks) did not enhance myelotoxicity and improved bone marrow recovery (97). In addition, GM-CSF or erythropoietin are frequently used to improve bone marrow function and recovery from toxicity in cisplatin regimens.

Radiation therapy has also been combined with gene therapy applications. Treatment of cultured C6 ($p53^+$) and 9L ($p53^-$) rat glioma cells with a Vaccinia virus carrying the p53 (VV-TK-p53) gene showed significantly reduced survival when combined with radiation in comparison to only radiation treatment (98). In nude mice bearing C6 tumors combination treatment with VV-TK-p53 and radiation resulted in significantly reduced tumor size. The p53 protein was expressed over 20 days based on immunohistochemical analysis. Examination of blood and spleen cells demonstrated significant splenomegaly, leukocytosis and increased DNA synthesis as well as response to mitogen. Overall, this study showed that the combination treatment was advantageous and could represent a promising strategy for the treatment of gliomas.

Table III
Clinical Trials: Use of delivery methods and vectors

Delivery vehicle	Number of trials
Viral vectors	
AAV	15
Adenovirus	171
Herpes simplex virus	5
Retrovirus	217
Poxvirus	39
Subtotal	447
Non-viral vectors	
Gene gun	5
Lipofection	77
Naked plasmid DNA	70
RNA	6
Subtotal	158
NA	25
Total	636

NA = data not available

Table IV
Clinical Trias: Status of phases

Phase	Number of trials	Number of patients
Phase I	420	1804
Phase I/II	134	914
Phase II	73	507
Phase II/III	5	NA
Phase III	4	251
Total	636	3476

NA = data not available

Clinical Trials

Various delivery systems for gene therapy applications have been established for clinical trials (99, Table III). The large majority of trials today have been conducted in North America (81.1%). European protocols represent 16.2%, Asian and Australian trials 1.4% and 0.5%, respectively, and the rest of the world, including multi-continental studies 0.8%. So far, the majority of trials have been set up applying viral vectors (70.3%). The most commonly used viral vector has been based on retroviruses (34.1%). More than 60% of the current trials are in Phase I and only 0.6% have reached Phase III (Table IV). Overall, more than 3000 patients have been treated by gene therapy. Due to the many trials in progress, only a few individual studies are described below.

The long-term follow-up of heavily pretreated individuals with recurrent ovarian cancer by p53 gene replacement using the adenoviral vector SCH 58500 followed by multiple cycles of platinum-based chemotherapy has been evaluated by Buller and coworkers (100, 101). The median survival of individuals who received multiple doses of SCH 58500 with chemotherapy was 12-13 months, compared to only 5 months for those treated with a single-dose of SCH 58500; this compared favorably to the 16-month median survival for individuals treated with paclitaxel at the time of initial recurrence of this disease and was more than double the 5-month survival seen with palliative radiotherapy or paclitaxel failure. In spite of adenoviral-induced inflammatory changes that made CT scans an invalid measure of response, intraperitoneal SCH 58500 was safe as a 5-day regimen at 7.5×10^{13} aden-

oviral particles per dose per day followed by intravenous carboplatin/paclitaxel chemotherapy and resulted in a significant reduction of serum tumor marker CA125 (100, 101).

Nine patients with recurrent and unresectable breast cancer and 9 patients with head and neck cancer were treated intratumorally with a liposomal formulation of the E1A gene using 3 β[N-(n´,n´-dimethylaminoethane)-carbamoyl] cholesterol/dioleoylphospha-tidyl-ethanolamine. No dose-limiting toxicity was observed in the 4 dose groups (15, 30, 60, and 120 μg DNA/cm of tumor and a maximally tolerated dose was not reached. E1A gene transfer was demonstrated in 14 of 15 tumor samples tested, and down-regulation of HER-2/neu was demonstrated in two of the five patients who overexpressed HER-2/neu at baseline. In 16 patients evaluable for tumor response, 2 had minor responses, 8 had stable disease, and 6 had progressive disease (102).

A phase I trial using AAV for the expression of the cystic fibrosis transmembrane conductor regulator (CFTR) was carried out in 25 CF patients with mild to moderate lung disease (103). Doses of 6×10^4 to 2×10^{12} particles were administered to one side of the nose and to the superior segment of the lower lobe of the right lung. Several adverse events were registered most of which were related to the endogenous CF lung disease and only one was suggested to be vector-related. The presence of vector DNA, evaluated by PCR technology, indicated that the gene transfer was very low in the nasal and bronchial epithelia, but higher in the lung. The study demonstrated that AAV can be used safely for gene delivery to the lung and additional studies will indicate the efficacy of this approach.

Allovectin-7, a plasmid DNA carrying the HLA-B7 and β2-microglobulin genes complexed with a cationic lipid mixture (76), was subjected to a phase II trial on patients with metastatic melanoma (78). The treatment was well tolerated and resulted only in mild to moderate adverse events (ecchymosis, pruritus and pneumothoraces). Tumor regression was observed in 18% of patients. One patient showed a complete response, in 3 patients partial responses were seen and 5 patients demonstrated minor responses. Six patients were alive 25.1 to 39.4 months after their first injection. The study showed that intratumoral Allovectin-7 administration is safe and can result in tumor regression and responses in overall disease. A phase III single tumor lesion study has also been conducted for Allovectin-7, which did not reach trial endpoints most likely due to to the low dosage (10 μg) (104).

Conclusions

The most prevalent problem in cancer therapy is the regrowth and metastasis of malignant cells after standard treatment with surgery, radiation, and/or chemotherapy. Additional hurdles arise from chemoresistance of tumors, toxicity of currently available chemotherapy regimens and inefficiency of cancer treatments especially for advanced stage of the disease. Tumor-targeted gene therapy methods promise to overcome some of these hurdles.

However, despite numerous preclinical and clinical studies, routine use of gene therapy for the treatment of human disease has not yet been a practice. It remains an important unmet need of gene therapy to create gene delivery systems that effectively target specific cells of interest in a subject while controlling harmful side effects. Gene therapy approaches have suffered from the inadequate transduction efficiencies of replication-defective vectors. Replication-competent vectors, particularly adenoviruses that cause cytolysis as part of their natural life cycle, represent an emerging technology that shows considerable promise as a novel treatment option, particularly for locally advanced or recurrent cancer. Especially promising are adenoviruses that selectively replicate in tumor cells that have shown encouraging preliminary results in clinical trials, especially in combination with chemotherapy. The genes of E1A of adenovirus and p53 show promising anticancer effects in clinical trials. The liposomal formulations of genes described here appear to overcome significant hurdles in gene therapy allowing their applications to a clinical setting. For example, one major problem is inability of systemic delivery of genes due to destruction of gene vehicles by circulating macrophages after intravenous or intraperitoneal injection. The liposomes can protect genes or viruses from immune responses and destruction. The second hurdle is posed at the cell membrane barrier; genes are not taken up readily by cells and usually they end up in lysosomal compartments where they get degraded. Liposomal encapsulation appears to be able to overcome this hurdle. Furthermore, encapsulation of viruses can lead to tumor targeting. For example, replication incompetent viruses such as Semliki Forest Viruses carrying therapeutic genes, like the human interleukin-12 gene, show promise in Phase I/II clinical trials as a cancer immunotherapy regimen.

Development of high technology pharmaceuticals against cancer based on gene therapy systems promise to elicit negligible side effects and to bring a major advancement and revolution in molecular medicine. For example, demonstration of tumor regression in animal xenograft models using virus-liposome combination systems and their feasibility in human clinical trials would lead to the development of novel pharmaceutical products based on virus-liposome complexes; this endeavor, although it may appear to come from a science fiction movie, is feasible and has a potential in the 60-billion dollar anticancer pharmaceutical market.

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